



## Hot Start Polymerase

Heat-activatable DNA polymerase for high specificity

*Thermus aquaticus*, recombinant, *E. coli*

Cat. No.	Amount
HS_100KU	100 kunits
HS_1000KU	1.000 kunits

**Unit Definition:** One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmol of dNTP into an acid-insoluble form in 30 minutes at 70 °C using hering sperm DNA as substrate.

**For general laboratory use.**

**Shipping:** shipped on blue ice

**Storage Conditions:** store at -20 °C

**Additional Storage Conditions:** avoid freeze/thaw cycles

**Shelf Life:** 12 months

**Form:** liquid

**Concentration:** 5 units/ $\mu$ l

### Description:

Hot Start Polymerase provides improved specificity and sensitivity when amplifying low-copy-number targets in complex backgrounds or when prolonged room-temperature set up is required. The polymerase activity is blocked at ambient temperature and switched on automatically at the onset of the initial denaturation. The thermal activation prevents the extension of nonspecifically annealed primers and primer-dimer formation at low temperatures during PCR setup. The polymerase is recommended for routine PCR applications (up to 4 kb fragment length), high throughput PCR or genotyping.

The Crystal Buffer system guarantees robust and reliable amplification results in almost all PCR applications. The buffer contains a well-balanced ratio of potassium-, ammonium- and magnesium-ions to ensure high specificity and minimal by-product formation without the need of additional optimization steps.

Ruby Buffer additionally contains gel loading buffer and an inherent red dye allowing the direct loading of the PCR product into the gel. The red dye allows an easy visual control during PCR set-up and in combination with the density reagent the direct loading of the reaction product into the gel.

The enzyme replicates DNA at 72 °C. It catalyzes the polymerization of nucleotides into duplex DNA in 5'→3' direction in the presence of magnesium. It also possesses a 5'→3' polymerization-dependent exonuclease replacement activity but lacks a 3'→5' exonuclease (proof-reading) activity.

### Activation step

Hot Start Polymerase requires no prolonged heating or denaturing step. The polymerase inhibiting aptamer is quickly released at the increased temperature of thermal cycling.

### Content:

#### Hot Start Polymerase (red cap)

5 units/ $\mu$ l aptamer-inhibited hot start polymerase in 20 mM Tris-HCl, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 % Tween-20, 0.5 % Nonidet P-40, 50 % (v/v) Glycerol, pH 8.0 (25°C)

#### Ruby Buffer (black cap)

10 x conc. complete PCR buffer containing 200 mM Tris-HCl, KCl,  $(\text{NH}_4)_2\text{SO}_4$  and 20 mM  $\text{MgCl}_2$ , red tracking dye and density reagent for gel loading

#### Crystal Buffer (green cap)

10 x conc. complete PCR buffer containing 200 mM Tris-HCl, KCl,  $(\text{NH}_4)_2\text{SO}_4$  and 20 mM  $\text{MgCl}_2$

### Assay Set-Up:

Before starting, vortex all components thoroughly to ensure homogeneity.

Prepare a premix for the number of assays you need according to the following protocol:



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comp.	cap	stock conc.	final conc.	1 assay @20 µl	1 assay @ 50 µl
PCR-grade Water	white			fill up to 10 µl	fill up to 30 µl
Ruby Buffer or Crystal Buffer	black or green	10x	1x	2 µl	5 µl
dNTP Mix / 10 mM #NU-1006	white	10 mM	200 µM	0.4 µl	1 µl
Hot Start Polymerase	red	5 units/µl	0.025 units/µl	0.1 µl	0.25 µl
primer mix or each primer		10 µM each primer	200 - 400 nM each primer	0.4-0.8 µl	1 - 2 µl
template /sample DNA				10 µl < 10 ng DNA	20 µl < 20 ng DNA

Select PCR tubes, stripes or plates as recommended for your cyclor model.

Aliquot premix into each well and add template DNA (or PCR-grade Water for negative controls).

### Cycling Conditions:

Spin down the tubes/plate briefly to remove bubbles and place them into the cyclor.

<sup>1)</sup>The annealing temperature depends on the melting temperature of the primers used.

<sup>2)</sup>The elongation time depends on the length of the fragments to be amplified. A time of 1 min/kb is recommended.